Bridging MOA and Measurement: Optimizing Cytokine Release Assays as a Mechanistic Potency Readout

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Published 10 November, 2025

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Abstract

The development of biologics, particularly in immuno-oncology (I-O) and cell therapy, has placed new demands on preclinical *in vitro* assays. Potency, a critical quality attribute (CQA), must be measured using assays that are quantitative and reflective of the drug's specific mechanism of action (MOA). For many I-O biologics, this MOA is the activation of an immune response, often measured via the release of key cytokines (IFN-γ, IL-2, TNF-α). This creates a significant "dual-challenge" for R&D teams: the assay is simultaneously a complex cell-based model *and* a quantitative immunoassay. This paper reviews the primary challenges in developing such assays, focusing on (1) aligning the biological cell model with the drug's MOA, (2) managing the analytical difficulties of quantifying cytokines in complex matrices, (3) applying a "fit-for-purpose" (FFP) framework to de-risk development, and (4) the foundational role of critical reagent management.

1. Introduction: The Potency Assay Mandate

For biotherapeutics, a potency assay is a quantitative measure of the drug's biological activity (Kumar, 2022). Regulatory agencies have consistently emphasized the need for potency assays to be based on the drug's specific mechanism of action (MOA) (Forge, 2025). This requirement, which is critical for later GxP compliance, creates a significant burden of proof during early preclinical (RUO) development.

For a growing class of therapies, including bi-specific antibodies, CAR-T cells, and checkpoint inhibitors, the intended MOA is the activation of an effector cell (e.g., T-cell) to kill a target cell and/or secrete signaling proteins. Assays measuring effector functions like Antibody-Dependent Cellular Cytotoxicity (ADCC) or T-cell-mediated cytokine release are therefore highly MOA-reflective (FUJIFILM, 2024).

However, using a secreted protein like IFN-γ as the potency endpoint creates a complex, two-part analytical problem. The R&D team must first develop a robust *biological system* that generates the signal and then develop a robust *analytical method* (e.g., ELISA) to quantify that signal, which is confounded by the very system that produced it. This paper reviews the key optimization challenges within this dual framework.

2. Aligning the Biological System with the MOA

The foundation of a relevant potency assay is its faithful reflection of the *in vivo* MOA. For a cytokine release assay, this involves building a functional co-culture model. This presents several key development challenges:

- **Cell Sourcing:** The choice of effector cells is paramount. Primary cells (e.g., PBMCs, purified T-cells) offer the highest biological relevance but suffer from significant donor-to-donor variability and limited culture lifespans. Conversely, immortalized cell lines (e.g., NK-92, Jurkats) provide a consistent, scalable, and stable reagent but may not fully recapitulate the drug's target biology (Kumar, 2022).
- **Model Complexity:** The assay must model the biological interaction. This often requires co-cultures of effector and target cells. Optimizing this system involves extensive characterization of variables such as effector-to-target (E:T) ratios, cell plating densities, and incubation times, all of which directly impact the magnitude and kinetics of the cytokine readout.
- **MOA-Specific Endpoints:** The chosen endpoint must align with the drug's function. While ADCC/ADCP assays measure cell lysis (a common endpoint), a cytokine release assay is designed to quantify the *signaling* that drives the immune response (FUJIFILM, 2024). This is a distinct biological event that must be proven to be the most relevant functional measure of the drug's potency.

3. Managing the Analytical Readout: The Matrix Effect

Once a biological model is established, the challenge shifts to the analytical quantification of its output: the secreted cytokine. This cytokine is not in a clean buffer; it is in a "diverse matrix" of conditioned cell culture supernatant (Bio-Rad, n.d.). This matrix can impact the assay in unpredictable ways, leading to an inaccurate signal.

High concentrations of media components, serum proteins, cell lysates, and potentially the therapeutic itself, can cause significant assay interference (Creative Proteomics, n.d.). This "matrix effect" is a well-documented challenge in immunoassay development (de Jager, 2009).

Common interferences include:

- Non-specific binding of matrix proteins to the assay plate or antibodies.
- Inhibition or enhancement of the antibody-antigen binding.
- Cross-reactivity of media components (e.g., phenol red, serum factors) with detection reagents.
- Competition from soluble receptors or endogenous binding proteins.

Failure to address the matrix effect during optimization leads to poor accuracy, low sensitivity, and high well-to-well variability in the immunoassay. This analytical noise can obscure the true biological signal, rendering the potency assay unreliable.

4. Deconvoluting Variability: A "Fit-for-Purpose" Framework

Biological systems are "inherently variable" (Abzena, 2024). When this is combined with a complex analytical readout, the resulting assay can be difficult to control. A "Fit-for-Purpose" (FFP) framework, distinct from full GxP validation, is an essential strategy for preclinical RUO development (SoCal, 2025).

For a cytokine release assay, the primary goal of FFP optimization is to **deconvolute variability**. When an assay run fails or a result is ambiguous, the team must be able to determine the source:

1. **Biological Variability:** Did the cells fail to activate? (e.g., poor cell health, incorrect E:T ratio, primary cell donor non-responsive).
2. **Analytical Variability:** Did the immunoassay fail? (e.g., matrix effect, bad antibody lot, reference standard degradation).

A "phase-appropriate method qualification" (K. A., 2024) focuses on characterizing and controlling these two sources of variance independently before combining them. This involves running analytical controls (e.g., spiking recombinant cytokine into the matrix) alongside biological controls (e.g., a positive control activator) to ensure both parts of the assay are functioning as expected.

5. The Foundation: Rigorous Critical Reagent Management

A primary driver of *both* biological and analytical variability is the management of critical reagents. An assay's long-term performance is entirely dependent on the quality and consistency of these components (O'Hara, 2014).

In a dual-challenge assay, the list of critical reagents is extensive:

- **Biological Reagents:** Master and working cell banks (for both target and effector lines), cryopreserved primary cells, and activation reagents (BioAgilytix, n.d.).
- **Analytical Reagents:** Capture and detection antibodies (often as matched pairs), enzyme conjugates, and the protein reference standard (O'Hara, 2014).

Lot-to-lot variability in any of these reagents can have "profound or unexpected effects on assay performance" (King, 2009). An FFP framework must include a robust plan for reagent life-cycle management, including initial screening, qualification of new lots against old lots, and monitoring for stability and performance trends over time.

6. Conclusion

Cell-based potency assays that rely on a cytokine release endpoint represent a convergence of complex cell biology and quantitative immuno-analytics. They cannot be developed in a silo; the

biological system and the analytical readout are inextricably linked.

Success in the preclinical (RUO) phase requires a holistic, "fit-for-purpose" optimization strategy. This approach must address the challenges of building an MOA-reflective cell model while simultaneously managing the analytical matrix effects of the immunoassay. By focusing on deconvoluting variability and implementing rigorous life-cycle management for all critical reagents, R&D teams can build a reliable data package that de-risks early-stage go/no-go decisions and establishes a clear, robust path for future GxP validation.

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